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# PULMONARY EFFECTS OF PYROTECHNICALLY DISSEMINATED TITANIUM DIOXIDE SMOKE IN RATS

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The Fast Obscurant Grenade (FOG) is a bursting type grenade that has been developed within the Family of Tactical Obscuration Devices to fulfill the small through medium area screening obscuration need. After the health hazards were analyzed for the currently used obscurant payloads, titanium dioxide ($TiO_2$) was chosen as the candidate smoke, while maintaining the necessary performance characteristics. Many studies have been performed evaluating the toxicity of inhaled $TiO_2$; however, most have evaluated long exposure times (i.e., 30 min) at low concentrations. For the current need supported by FOG, elevated concentrations for short exposure times would be the predominant operational scenario for inhalation exposures to $TiO_2$ smoke. Acute and repeat exposures are therefore possible as maneuvers are performed in confined areas and in close proximity to the dissemination source. The current study evaluated clearance of the smoke material from the respiratory system, as well as other biological effects. Groups of rats were exposed for 10 min to high concentrations of smoke generated from the FOG. Broncheoalveolar lavage, histopathology, particle size analysis, and chemical characterization of the aerosol were performed to assess the toxicity of the inhaled smoke.

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## PREFACE

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# PULMONARY EFFECTS OF PYROTECHNICALLY DISSEMINATED TITANIUM DIOXIDE SMOKE IN RATS

## 1. INTRODUCTION

Recently, the U.S. Army Chemical School and PM Obscuration performed an obscuration Mission Area Analysis (MAA) to identify the current critical uses of smokes/obscurants for the military.[1] A Functional Needs Analysis (FNA) was performed to determine operational capability gaps and limitations in the obscuration mission.[2] One of the capability gaps identified by the FNA was found in the small to medium obscuration area, where rapid short duration effects were needed.[3] Small to medium area obscuration is defined as restrictive terrain such as urban structures (i.e., 12' x 12' x 12' rooms) or smaller confined open terrain.[4] The Family of Tactical Obscuration Devices (FOTOD) was developed as a solution in response to this FNA. Other aspects of this FNA include the development of an operationally safe item that was toxicologically and environmentally friendly.[4]

The Fast Obscurant Grenade (FOG) is a burster grenade that has been developed within the FOTOD to fulfill the small through medium area screening obscuration need as defined by the FNA. Several materials have been considered as possible candidates for smoke payloads including white/red phosphorous, brass, carbon fibers and titanium dioxide ($TiO_2$). According to the documented short-term public emergency guidance levels and repeated public exposure guidance levels published by the National Research Council's Committee on Toxicology,[5] $TiO_2$ has less hazardous threshold levels as compared to these other smoke payloads. In addition, many of these other payloads produce high temperature flames during dissemination and would therefore be considered a safety hazard when used in small confined areas. $TiO_2$ was therefore chosen as the candidate smoke, while maintaining the necessary performance characteristics for satisfying the small to medium area screening obscuration need. Many studies have been performed evaluating the toxicity of inhaled titanium dioxide;[6,7] however most have evaluated longer exposure times (i.e., 30 min) at lower concentrations. For the current need supported by the FNA, elevated concentrations for short exposure times would be the predominant operational scenario for inhalation exposures to $TiO_2$ smoke as produced through FOG disseminations.

Acute (single) and repeat (multiple) exposures are possible as maneuvers are performed in confined areas and in close proximity to the dissemination source. As such, the current study will evaluate groups of rats exposed to high concentrations of smoke for acute and repeat inhalation exposures for 10 min. The toxicity of the inhaled smoke will be assessed through particle size analysis and chemical characterization of the aerosol. Additionally, clearance of the smoke material from the respiratory system as well as other biological effects will be evaluated through broncheoalveolar lavage (BAL) and histopathology analyses.

## 2. MATERIALS AND METHODS

### 2.1 Materials.

#### 2.1.1 FOGs.

Thirty FOGs (Table 1) were built by the Pyrotechnics and Production Team, Engineering Directorate, ECBC and stored at the Ammunition Storage Facility until time of testing. Each individual grenade was filled with approximately 255 grams of dry packed $TiO_2$ and was contained within an individual fiberboard tube.[8] The $TiO_2$ (purity 97%) used in the grenades is the chloride process, rutile grade (TRONOX® CR-470) formulated by Tronox Inc. (Hamilton, MS).[9] The remaining 3% was analyzed by Inductively Coupled Plasma and Energy Dispersive Spectroscopy and found to primarily contain alumina ($Al_2O_3$) and smaller percentages of silica ($SiO_2$).[10] According to the manufacturer, the material does not contain any Class I or Class II Ozone Depleting Substances, and contains hydrophobic properties that result in excellent dispersability and performance at high temperatures.[9] Each grenade also contained 8 grams of burster mix consisting of Potassium Perchlorate (Grade A, Class 4), Aluminum (ASTM D962 Type 1, Class A), and Pentaerythritol (98% pure, <#60 sieve).[8] On days of testing, grenade(s) were transported from the Ammunition Storage Facility to the ECBC pyrotechnic testing chamber. Figure 1 shows a FOG received for testing.

Table 1. FOGs Tested at ECBC

| | |
|---|---|
| NSN | 1330-00-D01-3475 |
| Lot Number | PLF06B000E001 |
| DOT Nomenclature | Ammunition, Smoke UN0016 |
| US Hazard Class | 1.3G |

Figure 1. FOG

2.1.2 Atomic Absorption Reagents and Materials.

The reagents, $TiO_2$, $Al_2O_3$, and $SiO_2$, were all bought from Sigma-Aldrich (St Louis, MO). Hydrofluoric acid (HF), sulfuric acid ($H_2SO_4$), phosphoric acid ($H_3PO_4$), and nitric acid ($HNO_3$) were bought from Fisher Scientific (Fair Lawn, New Jersey). The silicon (Si) atomic absorption (AA) standard (1000 ppm $H_2O$/0.04% HF) was bought from Perkin Elmer, titanium (Ti) AA standard (984 ppm in water) from Aldrich (St Louis, MO), and aluminum (Al) (1000 ppm in 0.05 M HCl) from Fluka (St Louis, MO). Water was obtained from an in-house filtration system (18 mΩ, reverse osmosis, ion filtration, carbon filtering). All standards were prepared in nalgene polypropylene volumetric flasks from Fisher Scientific.

2.2 Experimental Design – FOG dissemination.

Initially, a vice clamp was placed on a 3 ft. high metal table and placed in the middle of the 20,000-L chamber. The grenade was inverted and clamped in the vice such that the spring loaded handle was clear of the vice jaws (Figure 2). The FOG was inverted for better support prior to dissemination and was raised off the floor to help simulate an initial 360° air burst. Chamber concentration would be maximized as immediate impaction of the smoke payload on the walls and/or floor of the chamber would be minimized. A nylon lanyard line was hooked to the pin, and fed through a small hole that had been drilled into a rubber stopper. The stopper was inserted into the wall of the 20,000-L chamber. The grenade was activated by taking up the slack on the line and pulling the lanyard. [11] Equilibrium was quickly achieved as the aerosol statically distributed through the chamber. For exposures involving the simultaneous dissemination of two grenades, the second grenade was also inverted,secured in a vice on the chamber floor and disseminated by pulling a second lanyard line.

Only certified ammunition/explosive personnel handled the FOG(s) during testing. The initial time ($t_0$) coincided with pulling the pin from the grenade(s); however, there was an approximate 0.5 second delay upon pulling the pin and dissemination of the FOG(s). Confirmation of dissemination was performed through audibly hearing the burst and visually observing the smoke through a chamber portal window. When two FOGs were used, they were disseminated within 3 sec of each other to audibly confirm dissemination of each. One minute was allowed to mix the smoke ($t_0$-$t_1$) before opening the valve that allowed for the smoke to be delivered from the 20,000-L chamber to the 500 L sampling chamber (Figure 3). Ten minute exposures were conducted from 1-11 min ($t_1$-$t_{11}$) after dissemination. At 11 min post dissemination, the three way valve between the chambers was closed to the 20,000-L chamber and opened for clean room air to be delivered to the 500-L chamber. The aerosol quickly dissipated from the 500-L chamber.

Chamber environmental parameters monitored during all of the tests were temperature, relative humidity, and airflow. During calibration experiments, chamber distribution within the 500-L chamber was also confirmed. Samples were drawn within the breathing zone of the animals from nine locations in the chamber (Figure 4).

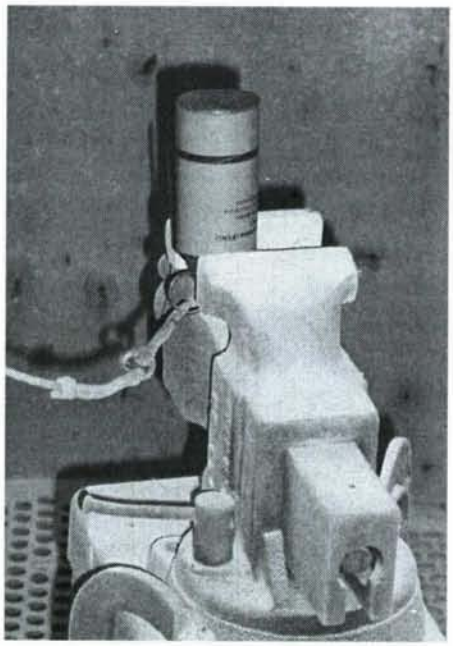

Figure 2. FOG Clamped in Vice Prior to Dissemination

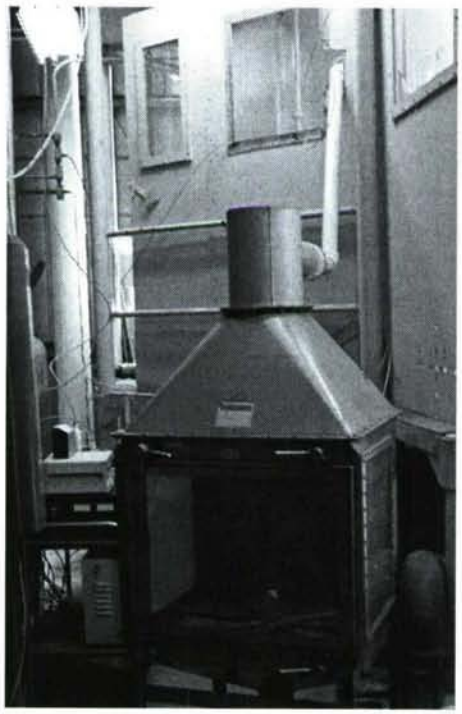

Figure 3. 500-L Exposure Chamber

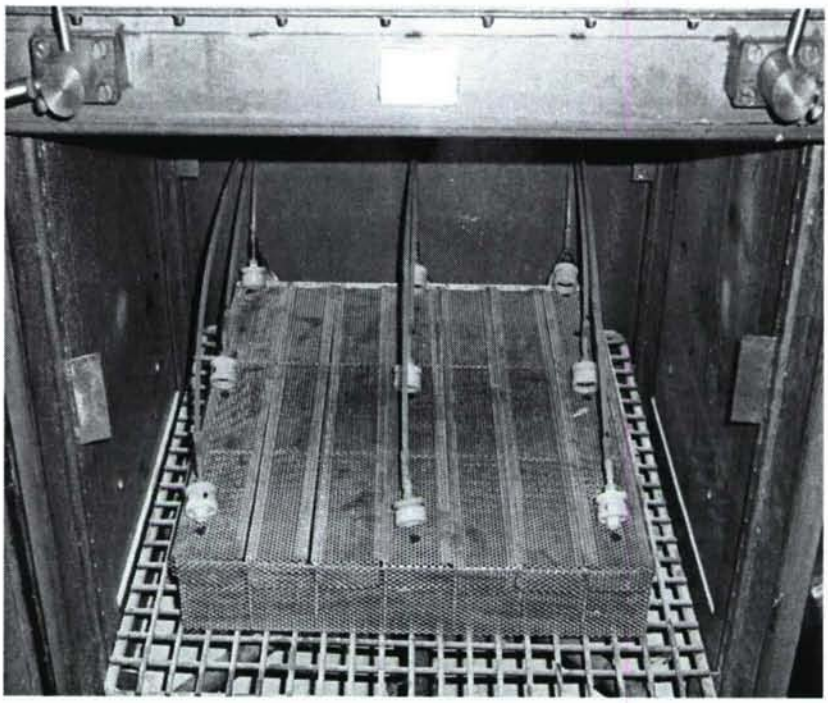

Figure 4. Filter Pad Locations to Monitor Chamber Distribution

2.3 Sampling and Monitoring Exposure Chamber Concentration.

2.3.1 500-L Chamber Concentration.

To determine the total aerosol concentration in the 500-L chamber produced from the FOG(s), two 25 mm HEPA A/E Glass Fiber Filter (GFF) pads (Gelman Scientific) were used to collect aerosol samples during the 10-min exposures. Vacuum pumps (Sierra Instruments) controlled with mass flow devices were used to collect samples from two of the locations that had previously been used to initially establish chamber distribution. Flows were set with a Gilibrator air flow calibration system (Scientific Instrument Services, Inc., Ringoes, NJ). Samples were drawn from the time of dissemination to the time when the chamber was cleared of aerosol (approximately $t_{15}$) at a flow rate of 1 Lpm. Additionally, one filter pad sample was taken from the 20,000-L chamber for 1 min ($t_1$-$t_2$) at a flow rate of 1 Lpm. Gravimetric analysis was subsequently performed on the resulting pads using a Cahn microbalance to determine the aerosol concentrations in the 20,000 and 500-L chambers.

2.3.2 Particle Size Collection.

Cascade impactors (Sierra Instruments, Monterrey CA) were used to monitor the particle size distribution of the generated smoke cloud. Sampling was collected for 1 min ($t_3$-$t_4$) at a flow rate of 7 Lpm (as specified by the manufacturer). GFF substrates were used to collect the particles on the stages. Gravimetric analysis using a Mettler MT5 microbalance was used to determine the mass collected on each stage of the impactor.

2.3.3 Exposure Chamber Concentration Profile (DustTrak™).

Real-time monitoring of chamber concentration was performed with the DustTrak™ Aerosol Monitor (Model 8520, TSI Inc. Shoreville, MN). Dilution and exposure chamber air were fed to the DustTrak™ to reduce the aerosol concentration to a level that could be reliably monitored by the DustTrak™ . The concentration profile (rise, equilibrium, and decay) and stability were recorded for all exposures. When the valve between the 20,000 and 500-L chamber was opened at $t_1$ minutes, the concentration in the 500-L chamber gradually rose until equilibrium was reached. This equilibration time is dependent on airflow and concentration. At the conclusion of the 10 min exposure ($t_{11}$), the three way valve between the chambers was closed to the 20,000-L chamber and opened for clean room air to be delivered to the 500-L chamber. Once the DustTrak™ returned to baseline, the animals were removed from the chamber.

2.3.4 Volatile Organic Combustion Products (VOCs).

Smoke vapor samples were drawn and collected onto 10 mm multibed sorbent tubes (CDS Dynatherm Inc., Oxford, PA)(Part Number AO-06-2731) packed with equal portions of Tenax-TA, Carboxen 1000, and Carbosieve S111. Three sorbent materials were used to assure that high and low molecular weight compounds with varying volatilities would be trapped. Prior to their use, all sampling tubes were conditioned at 300 °C for 30 min with nitrogen flows of 50 mL/min. Control tubes were also drawn from the chamber to perform background analyses. During the dissemination, two tubes were used to sample the smoke cloud for VOCs from separate locations in the 500-L chamber. To prevent aerosols from passing into the tubes, GFF pads were attached to the front portion of each tube. Vacuum flows through the tubes were recorded with the pads attached to adjust for any minor resistance that could be introduced from the pads. Rates were set with mass flow controllers and checked against a separate external flow-measuring device (Gilibrator). Samples were drawn for 10 min ($t_1$-$t_{11}$) at a rate of 1 Lpm.

Thermal desorption Gas Chromatography Mass Spectrometry (GC/MS) was used to analyze for VOCs collected on the tubes following NIOSH Method 2549.[12] The thermal desorption system was a CDS Analytical ACEM 900 system and the GC/MS system was an Agilent 6890 GC equipped with a 5973 mass selective detector. Prior to injection onto the GC/MS, all samples were concentrated within the thermal desorption system onto a trap (CDS Dynatherm Inc., Part Number AC-06-5223) containing the same three sorbents that were used during field collection.

2.3.5 Inorganic Gas Collection.

Gas samples were manually drawn from the chamber at $t_6$ minutes using a two-liter gastight syringe (Hamilton). Two consecutive draws were pulled with each sample collecting approximately 1.5-2 L. Each air sample drawn was transferred into an inert Tedlar bag (SKC, Eighty Four, PA) for subsequent analyses. Samples were withdrawn from the bags onto compound specific detector tubes (Kitegawa, Schaunberg, IL) using Matheson portable gas sampling pumps (Model 400). Concentrations were recorded by monitoring the

colorimetric change observed on the sorbent material. Nitrogen oxides ($NO_x$), phosphine ($PH_3$), hydrogen fluoride (HFl), ammonia ($NH_3$), carbon monoxide (CO), carbon dioxide ($CO_2$), hydrogen cyanide (HCN), formaldehyde (HCHO), sulfur dioxide ($SO_2$) and hydrogen chloride (HCl) were all analyzed during the study. Table 2 lists the manufacturer's (Kitegawa) tube part numbers along with their respective measuring range.

Table 2. Kitegawa Part Numbers and Measuring Ranges for Tested Inorganic Gases

| Inorganic Gas | Manuf Part Num | Meas Range(ppm) |
|---|---|---|
| | | |
| $NO_x$ | 8014-175U | 0.5-30 |
| $PH_3$ | 8014-121SD | 0.25-10 |
| HFl | 8014-156S | 1-30 |
| $NH_3$ | 8014-105SC | 5-130 |
| CO | 8014-106S | 10-250 |
| $CO_2$ | 8014-126SF | 100-4000 |
| HCN | 8014-112SB | 0.5-100 |
| HCHO | 8014-171SB | 1-35 |
| $SO_2$ | 8014-103SE | 0.25-10 |
| HCl | 8014-173SB | 0.4-40 |

2.3.6 Metal Analysis – Extraction Efficiencies.

Extraction efficiencies for the principal metals contained in the $TiO_2$ payload is accomplished through quantitative Atomic Absorption Spectroscopic (AAS) analysis. The metals in the payload are in oxidized forms and must be reduced to their elemental forms (i.e., no oxides ) before analysis by AAS. This is accomplished through acid digestion and microwave extraction to reduce the metal oxides. The three primary metals analyzed from the $TiO_2$ payload were Ti, Al, and Si. The first step was to develop the acid digestion and microwave extraction methodologies for achieving high extraction efficiencies for the primary metal oxides ($TiO_2$, $Al_2O_3$, and $SiO_2$) The extraction instrumentation was an MDS 81D model purchased from CEM (Mathews, NC).

The metal oxide standards were used to determine the acid digestion and microwave digestion parameters for each metal with >90% extraction efficiency. Solid $TiO_2$, $Al_2O_3$, and $SiO_2$ material were weighed into microwave extraction vessels from CEM. After the extraction procedures, each vessel was rinsed three times with deionized water, transferred to their respective volumetric flask, and brought up to volume. Ti extraction was accomplished by adding 10 mL HF and 5 mL of $HNO_3$ to the vessels. The microwave extraction was performed at 70% power for 25 min followed by a cooling period. Samples were transferred to separate 100 mL polypropylene volumetric flasks, each containing 2 mL of an ion suppression solution (1.3 M Potassium Chloride 0- KCl). For Al, extraction was initiated by adding 5 mL each of $H_2SO_4$ and $H_3PO_4$ to the vessels. The microwave extraction was performed at 70% power for 6 min and the samples were transferred to separate 50 mL polypropylene volumetric flasks. For Si, extraction was initiated by adding 15 mL of HF and 3 mL of $H_3PO_4$ to the vessels.

The samples were allowed to sit overnight in the hood and subsequently transferred to separate 50 mL polypropylene volumetric flasks.

2.3.7 Metal Analysis ($TiO_2$, $Al_2O_3$ and $SiO_2$) – Sampling and Analysis (500-L Chamber).

The AAS was used to analyze the material collected from the disseminations. The instrumentation was a 2380 Series Perkin Elmer AA (Boston, MA) with a nitrous oxide/acetylene burner head. Hollow cathode lamp elements specific for each metal were used. The primary wavelengths were used for each element and were 364.4, 250.9 and 308.3 nm for Ti, Al and Si, respectively. Methodology guidance was followed according to CEM and AA literature.

To determine the Ti concentration, two 25 mm A/E GFF pads were used to collect aerosol samples during the 10-min exposures. Samples were drawn with a calibrated vacuum pump (Sierra Instruments) for 10 min ($t_1$-$t_{11}$) at a flow rate of 1 Lpm. Additionally, one filter pad sample was taken from the 20,000-L chamber for 1 min ($t_1$-$t_2$) at a flow rate of 1 Lpm. These were the same pads used to assess concentration from the 500 and 20,000-L chambers. The GFF pads were placed in petri dishes and stored in a dessicator. Post exposure weights for the pads had previously been recorded during the assessment of chamber concentrations. Samples were subsequently prepared for analysis (Section 2.3.6).

The GFF pads could not be used to analyze for Al and Si concentrations due to the presence of these metals inherently present in the pads. Therefore, they were determined directly from collected FOG "fallout" material collected from the main chamber. The fallout was collected on four large watch glasses placed in the chamber prior to dissemination. Large amounts were used in the extraction methodology to assure the detection of Al and Si by AAS. Separate portions of the "fallout" were subsequently weighed and prepared for analysis (Section 2.3.6).

2.4 Animal Exposures.

2.4.1 Animal Model.

Young adult male Sprague-Dawley rats (8-10 weeks) were obtained from Charles River Laboratories, Inc., (Wilmington, MA). The animals were identified by tattoo on the tail and housed individually in plastic shoebox cages. They were placed on racks in an American Association for Accreditation of Laboratory Animal Care (AAALAC) accredited facility. Prior to exposure, the animals were housed for a minimum of 3 days for quarantine. Ambient conditions were maintained at 70 ± 5° F, 30 - 70% relative humidity, and a 12:12 hr light-dark cycle. Rats were provided with certified laboratory rat chow and filtered house water *ad libitum*, except during exposure.

2.4.2 Whole-Body Inhalation Exposures.

Acute, 2-day repeat and 4-day repeat inhalation exposures to one disseminated FOG were performed. Additionally, acute and 4-day repeat exposures to the smoke produced from two FOGs were also performed. Prior to exposure, animals were placed into separate compartments of a metal cage. For each whole body exposure, 20 rats were used and the exposure duration was 10 min. Ten of the animals were used to assess the respiratory and biological effects of the inhaled smoke at 24-hr post exposure, while the other 10 animals were used to assess 14-day effects. Of the 10 rats used for 24-hr post exposure effects, 5 were used for BAL and 5 were used for histopathology. An identical breakdown was necessary for the 10 animals used to assess 14-day effects. During chamber operations, the airflow through the chamber was kept constant. The concentration-time profile generated with this type of chamber is described in a review by MacFarland(1987).[13] His definition of exposure was the one used in this study: the interval from the start of test material introduction into the chamber to the time-point when the test material supply is stopped. Animals were removed after the material had exited the 500-L chamber.

2.4.3 Observation of Toxic Signs.

Dysapnea, tachypnea, and flaring nostrils were some of the conditions used to help monitor respiratory ventilation of the animals. Labored breathing is normally characterized by gasping and larger than normal chest expansion to overcome airflow restrictions. Due to the high smoke concentrations present in the chamber, visual observations of respiratory distress were not possible during exposures. However, observations were made during the post exposure period.

2.4.4 BAL Evaluations.

Following exposure, the animals reserved for BAL were anesthetized with urethane using a 21 gauge needle at an initial dose of 1.5 g/kg body weight. Once anesthetized, the lavage procedure was commenced. The lung washing procedure consisted of instilling a calculated volume of normal saline (0.015 mL/g body weight) into the lung. The instilled saline was not withdrawn until a slight pressure was detected on the syringe plunger. Three lavage washes were repeated. The recovered lavage fluid was centrifuged (300 g) at room temperature for 10 min and pooled at 4 °C. After centrifugation, the supernatant was removed from the cell pellet. The pellet was resuspended in 1 mL of 50% bovine serum albumin and total cell counts were taken on a ZBI Coulter Counter (Beckman Coulter, Inc., Fullerton, CA). A differential cell count was taken on a hemocytometer and cell viability determined using the trypan blue dye exclusion test. The supernatant lavage fluid was assayed for the pro-inflammatory cytokines tumor TNFα and IL-1 using Enzyme Linked Immunosorbent Assay kits purchased from R&D Systems Inc. (Minneapolis, MN)

2.4.5 Histopathological Evaluations.

The animals were anesthetized using a 21 gauge needle to deliver an initial dose of 1.5 g/kg body weight urethane. The anesthetized animals were exsanguinated by cutting the

abdominal aorta and draining the lungs of blood. The lungs were collapsed by puncturing the diaphragm. The lungs were removed, and fixed under pressure, through a tracheal puncture with a solution of 10% neutral buffered formalin under 30 cm $H_2O$ pressure. The trachea was closed and the lungs were submerged in the fixative solution for 24 hr at a depth of 30 cm at room temperature. Tissues were sent to the U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM) for analysis under their pathology contract. Up to 8 tissue blocks (3 to 5 mm) were sectioned by the contractor from the various lobes of the lung.[14]

2.4.6 Data Analysis.

A statistical "decision tree" process was used to define the parameters of each exposure group and to determine if the defined groups were statistically different from each other. This process will evaluate the pulmonary and histopathological changes at 24-hr and 14-day postexposure. First, Bartlett's Test for homogenicity of variance was used as a check of the assumption of equivalent variances, followed by an Analysis of Variance (ANOVA). Non-parametric, homogeneous data was analyzed by the Kruskal-Wallis non-parametric ANOVA. Finally, Dunnett's Test was used on parametric homogeneous data to identify significantly different groups.

# 3. RESULTS

## 3.1 Chamber Concentration.

The six exposure groups evaluated during the study were acute, 2-day repeat and 4-day repeat for one FOG and acute and 4-day repeat for two FOGs.

### 3.1.1 Exposure Chamber Concentration – One FOG Dissemination.

Exposure chamber concentration data is summarized in Table 3. For the acute, 2-day repeat and 4-day repeat exposures combined, the mean total particulate concentration in the 500-L chamber was 1,854 $mg/m^3$ with a Standard Deviation (SD) of 125 $mg/m^3$ and a % Relative Standard Deviation (RSD) of 7. For the acute exposure only, the mean total concentration was 1,700 $mg/m^3$. For the 2-day repeat exposure, the mean total particulate concentration was 1,774 $mg/m^3$ with a SD of 117 $mg/m^3$ and a RSD = 1. For the 4-day repeat exposure, the mean total particulate concentration was 1,894 $mg/m^3$ with a SD of 140 $mg/m^3$ and a RSD = 7. The mean total particulate concentration in the 20,000-L chamber was 4,211 $mg/m^3$ with a SD of 316 $mg/m^3$ and a RSD = 7.

### 3.1.2 Exposure Chamber Concentration – Two FOG Disseminations.

Exposure chamber concentration data is summarized in Table 3. For the acute and 4-day repeat exposures combined, the mean total particulate concentration in the 500-L chamber was 3,649 $mg/m^3$ with a SD of 102 $mg/m^3$ and a RSD = 3. For the acute exposure only, the mean total concentration was 3,697 $mg/m^3$. For the 4-day repeat exposure, the mean

total particulate concentration was 3,638 mg/m$^3$ with a SD of 1,137 and a RSD = 3. The mean total particulate concentration in the 20,000-L chamber was 9,757 mg/m$^3$ with a SD of 517 mg/m$^3$ and a RSD = 5.

Table 3. Summary of $TiO_2$ Exposure Concentrations in Male Rats – 500-L Chamber

| FOGs Disseminated | Exposure Type | $TiO_2$ Concentration (mg/m$^3$) | Exposure Date |
|---|---|---|---|
| 1 | Acute | 1700 | 6/13/06 |
| | Repeat – 2Day (Day 1) | 1782 | 6/28/06 |
| | Repeat – 2Day (Day2) | 1766 | 6/29/06 |
| | Repeat – 4Day (Day 1) | 1917 | 7/10/06 |
| | Repeat – 4Day (Day 2) | 1848 | 7/11/06 |
| | Repeat – 4Day (Day 3) | 2072 | 7/12/06 |
| | Repeat – 4Day (Day 4) | 1738 | 7/13/06 |
| 2 | Acute | 3697 | 7/31/06 |
| | Repeat – 4Day (Day 1) | 3730 | 7/31/06 |
| | Repeat – 4Day (Day 2) | 3477 | 8/1/06 |
| | Repeat – 4Day (Day 3) | 3705 | 8/2/06 |
| | Repeat – 4Day (Day 4) | 3639 | 8/3/06 |

3.2 Particle Size Collection.

As the aerosol is drawn through the impactor, the larger particles are deposited on the first few substrates (lower numbers) and the smaller particles are deposited on the higher numbered substrates. The final stage is a filter to collect the remaining particulates that are not separated out among the other substrates. Table 4 shows an example of the raw particle size data that was accumulated from the dissemination of one FOG from the 500-L chamber. The cut off diameters ($D_P$) of the stages are shown and range from 18 μm to 0.32 μm. The sample weights for each stage were calculated by subtracting the tare weights from their respective gross weights. The cumulative total for the stages is calculated by summing the sample weights from the current and preceding stages. The respirable mass percentages were calculated using the current American Conference of Governmental Industrial Hygienists (ACGIH) model. Nearly 60% of the aerosol was collected on the middle stages (4-6) of the impactor, 25% on stages 3 and 7 and the remaining 15% collected on stages 1,2,8, and Final. This distribution of the mass fractions observed was characteristic for all of the particle size analyses. Figure 5 is the graphical representation of the data in Table 4. The mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD, $\sigma_g$), and correlation coefficient ($r^2$) for this dissemination were calculated from the regression data and are also shown in Figure 5. For the disseminations of one FOG where particle size analysis was performed, the mean values for MMAD, $\sigma_g$, and respirable mass percentage from the 500-L chamber were 2.20 μm, 2.64 and 69.6 %, respectively.

Table 4. Particle Size Data after Collection of Aerosol with Cascade Impactor

| stage | Dp | Filter tare wt. | Filter gross wt. | Sample Wt. | Cumulative Total | Respirable Mass |
|---|---|---|---|---|---|---|
| | (μm) | (mg) | (mg) | (mg) | (mg) | (%) |
| F | na | 119.4 | 119.7 | 0.3 | 0.3 | 2.2 |
| 8 | 0.32 | 106.2 | 106.9 | 0.7 | 1.0 | 5.9 |
| 7 | 0.53 | 99.5 | 101.0 | 1.5 | 2.5 | 12.0 |
| 6 | 0.95 | 100.5 | 103.7 | 3.2 | 5.7 | 25.7 |
| 5 | 1.7 | 104.2 | 106.1 | 1.9 | 7.6 | 13.6 |
| 4 | 2.65 | 100.7 | 102.8 | 2.1 | 9.7 | 8.9 |
| 3 | 4.4 | 106.1 | 107.8 | 1.7 | 11.4 | 0.9 |
| 2 | 11 | 100.6 | 101.1 | 0.4 | 11.8 | 0.0 |
| 1 | 18 | 99.3 | 99.6 | 0.3 | 12.1 | 0.0 |
| | | | | | | 69.2 % |

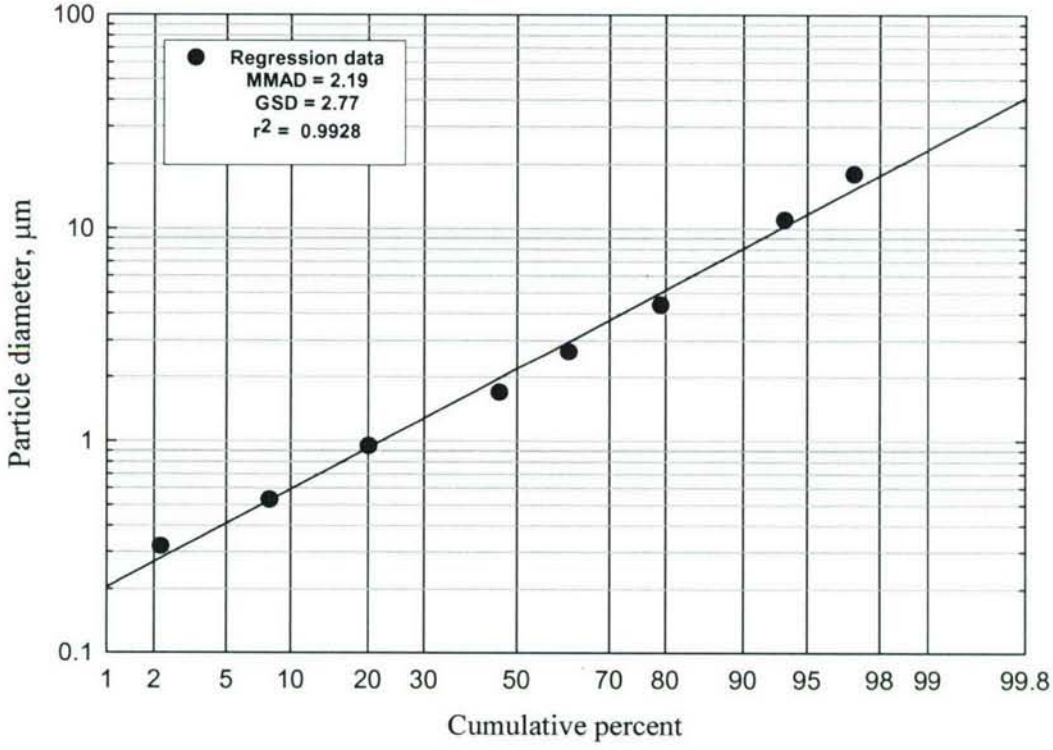


Figure 5. Particle Size Distribution

The particle size analysis (MMAD, $\sigma_g$, and respirable mass percentage ) from the 500-L chamber for the dissemination of two FOGs in the 20,000-L chamber was calculated to be 3.17 μm , 2.30 and 62.3 %. Nearly 65% of the aerosol was collected on stages 4-6, 23% on stages 3 and 7 and the remaining 12% collected on stages 1, 2, 8, and Final.

3.3 Exposure Chamber Concentration Profile (DustTrac™).

Figure 6 is a typical exposure profile for one of the disseminations that was conducted during the study. As the three way valve was opened between the chambers and closed for clean room air, the DustTrac™ showed a clear, sharp rise from baseline before reaching equilibrium. With the airflow going through the chamber, the time for equilibration was approximately 2.1 min. At the conclusion of the exposure, the three way valve between the chambers was closed to the 20,000-L chamber and opened for clean room air to be delivered to the 500-L chamber.

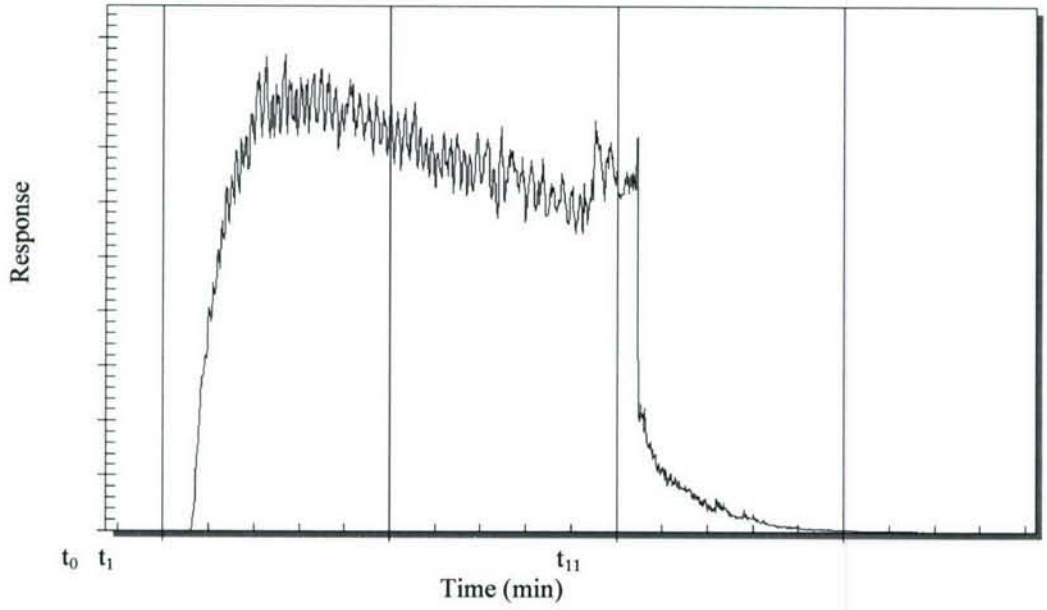


Figure 6. Real Time Monitoring of Concentration Stability Using DustTrac™ System

3.4 VOCs.

The VOCs detected during the study are shown in Figure 7. The chromatogram designated as 'blank tubes' shows those peaks, which are inherently present on the tubes, while the chromatogram designated as 'air blank' refers to those peaks that were collected from the 500-L chamber prior to dissemination. Both of these were background subtracted from the VOCs detected during the dissemination (chromatograms #1-6) prior to determining the presence of compounds. There were few similarities observed among the dissemination chromatograms. To conclude that the mass spectral fragmentation pattern of an individual peak found in the sample matches the compound fragmentation pattern in the spectral library, a qualifying index $\geq$ 80% was required. At most, some compounds were detected in three of the chromatograms but their respective mass spectrometry fragmentation patterns possessed qualifying indices less than 60 %. Most of the compounds seen were only observed in one chromatogram and with very low qualifying indices (<50%). Most of the compounds from the disseminations were long straight

chained hydrocarbons and had retention times of 10 min or greater. There were no polynuclear aromatic hydrocarbons detected in the disseminated smoke.

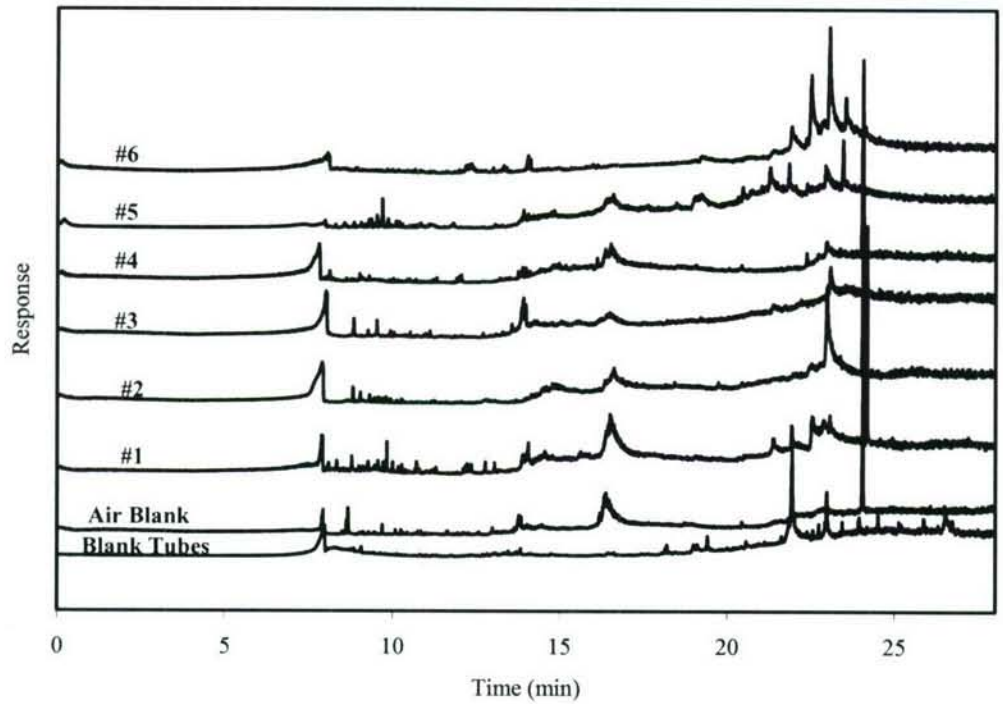


Figure 7. GC/MS Chromatograms of Air Samples Collected from FOG Disseminations

3.5 Inorganic Gases.

Inorganic gases were collected during disseminations for one and two FOG devices. CO, $NO_x$ and $CO_2$ were the only gases observed above their detection limits on the colorimetric detector tubes. After subtracting the background, $CO_2$ levels were found to be approximately 200-250 ppm for one FOG and 350 ppm for two FOGs. $NO_x$ levels were 0.25 ppm (for one or two devices) and CO levels were observed to be 10 ppm for one FOG device and nearly 20 ppm for two FOG devices. No other inorganic gases were detected in the organic smoke.

3.6 Metal Analysis.

3.6.1 Metal Analysis – Standards and Calibration.

Calibration standards were prepared from stock solutions procured from Sigma Aldrich. For Ti, concentrations of 150, 100, 50, 10, and 5 ppm Ti were prepared. To each Ti standard, 10 mL of HF, 5 mL of $HNO_3$ and 2 mL of 1.3 M KCl were added. For Al, concentrations of 150, 100, 50, 10 and 5 ppm Al were prepared. To each Al standard, 5 mL of $H_3PO_4$ and 5 mL of $H_2SO_4$ were added. For Si, concentrations of 500, 200, 100, 50, and 10 ppm Si were prepared. To each Si standard, 10 mL of HF and 3 mL of $HNO_3$ were added. The limits of detection were determined to be 4, 5, and 10 ppm for Ti, Al and Si, respectively. GFF pad

blanks were prepared to assure the absence of interferents for $TiO_2$. Figures 8-10 show the calibration curves for the three elements.

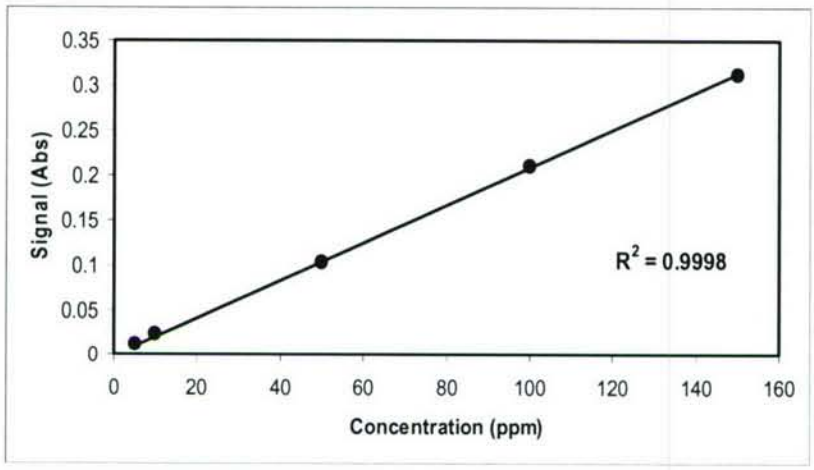


Figure 8. AA Calibration Curve for Ti

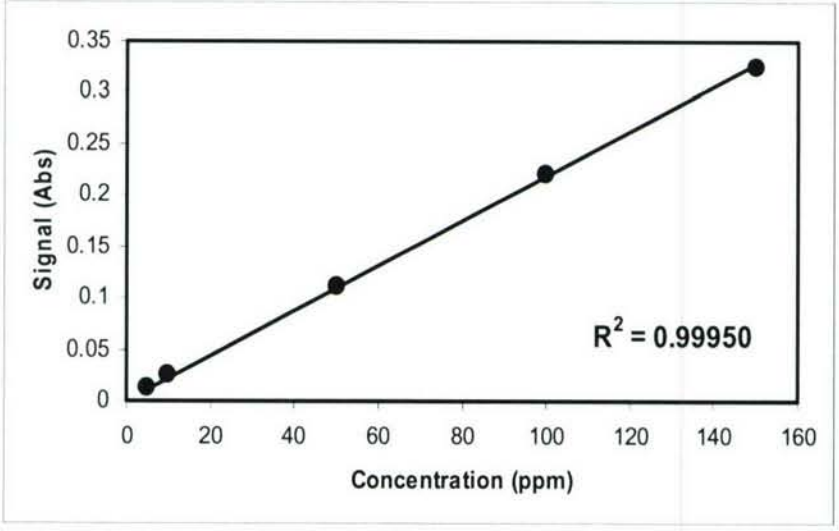


Figure 9. AA Calibration Curve for Al

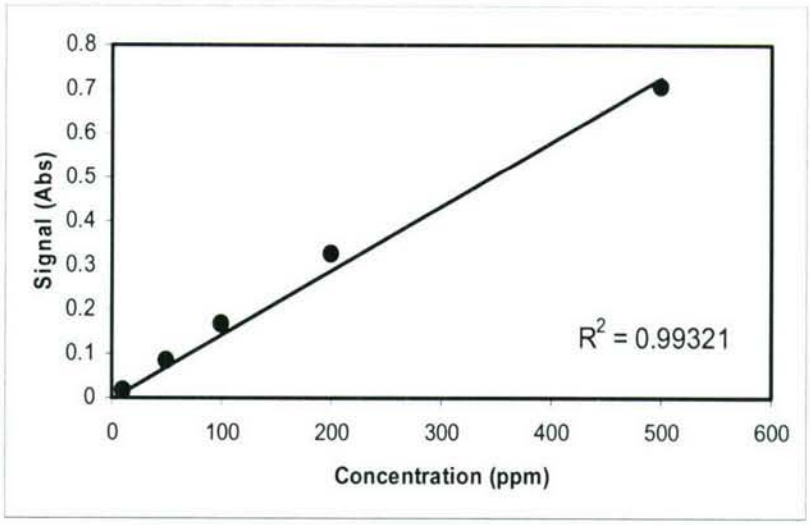


Figure 10. AA Calibration Curve for Si

3.6.2 Metal Analysis – Extraction Efficiencies.

Table 5 gives the percent recovery of Ti from the $TiO_2$ standard. The percent recovery of titanium from $TiO_2$ was determined by weighing out different concentrations of standard and extracting the subsequent Ti metal from its oxide using the microwave extraction and acid digestion methodology. Under the conditions determined for the extraction of Ti and $TiO_2$, the percent recovery was determined to be >95%. The percent recovery of Ti by AA is discussed below from the data given in Table 5.

An 8.36 mg sample of $TiO_2$ is weighed and placed into a vessel. Using stoichiometric calculations, the mg of Ti in the sample was determined to be 5.01 mg. Following microwave extraction and acid digestion, the extracted solution of Ti was diluted to 100 mL in a PTFE volumetric flask giving a nominal (theoretical) concentration of Ti as 50.12 ppm. The AA measured an experimental concentration of Ti as 50.00 ppm giving a percent recovery of 99.76% for Ti from $TiO_2$. The above calculations for Ti were also used to calculate the percent recoveries for Al and Si.

For all of the weighed $TiO_2$, $Al_2O_3$ and $SiO_2$ samples, the average experimental percent recoveries and RSD values for Ti, Al and Si were 98.16 % 1.63, 95.23 %, 2.03 and 104.90 % 3.37, respectively. Individual recoveries are shown in Tables 5-7.

Table 5. Percent Recovery of Ti from $TiO_2$

| Sample Number | $TiO_2$ (mg) | Ti (mg) | Nominal Ti conc (ppm) | Experimental Ti conc (ppm) | % Recovery |
|---|---|---|---|---|---|
| 1 | 8.36 | 5.01 | 50.12 | 50.00 | 99.76 |
| 2 | 14.05 | 8.42 | 84.23 | 81.34 | 96.57 |
| 3 | 2.6 | 1.56 | 15.59 | 15.30 | 98.15 |

Table 6. Percent Recovery of Al from $Al_2O_3$

| Sample Number | $Al_2O_3$ (mg) | Al (mg) | Nominal Al conc (ppm) | Experimental Al conc (ppm) | % Recovery |
|---|---|---|---|---|---|
| 1 | 0.38 | 0.20 | 4.02 | 3.75 | 93.12 |
| 2 | 0.58 | 0.31 | 6.14 | 5.99 | 97.61 |
| 3 | 0.96 | 0.51 | 10.16 | 9.74 | 95.83 |
| 4 | 0.6 | 0.32 | 6.35 | 5.99 | 94.36 |

Table 7. Percent Recovery of Si from $SiO_2$

| Sample Number | $SiO_2$ (mg) | Si (mg) | Nominal Si conc (ppm) | Experimental Si conc (ppm) | % Recovery |
|---|---|---|---|---|---|
| 1 | 21.4 | 10.00 | 200.06 | 220.28 | 110.11 |
| 2 | 31.9 | 14.91 | 298.23 | 309.79 | 103.88 |
| 3 | 28.3 | 13.23 | 264.57 | 270.63 | 102.29 |
| 4 | 20.2 | 9.44 | 188.85 | 195.11 | 103.32 |

3.6.3 Metal Analysis – Aerosol Concentration.

From the initial determination of the composition of the $TiO_2$ payload, a large percentage (>95%) of the aerosol concentration (mg/m3) was $TiO_2$. However, the smoke payload, does contain $Al_2O_3$ and $SiO_2$, which even at low concentrations (<0.5 ppm), can be considered toxic. With this in mind, it became important to determine the aerosol concentration of not only $TiO_2$, but also $Al_2O_3$ and $SiO_2$. Aerosol concentrations for $TiO_2$ samples were determined directly from the GFF pads by using the total weight of $TiO_2$ calculated from the AA following microwave and acid digestion. Tables 8-9 provide the aerosol concentration from the 500 and 20,000-L chambers for $TiO_2$ collected on the GFF pads, respectively. A sample calculation for the aerosol concentrations of $TiO_2$ collected from the exposure chamber is discussed.

Column 2 is the experimental concentration (ppm) of Ti that was recovered from the AA. This value was multiplied by the volume of sample (0.1 l) to give the mg of Ti on the pad (Column 3). Using stoichiometric calculations, the mg of Ti determined by AA was converted to the corresponding mg of $TiO_2$. The total weight collected on GFF pads from the 500-L chamber is given in column 5. By dividing the experimental $TiO_2$ (column 4) by the total weight on the GFF pad (column 5), the percent of $TiO_2$ on the GFF pad is calculated and given in column 6. For the filter pad samples collected from the 500-L exposure chamber, it was observed that a majority of the total particulate aerosol collected on the pad was $TiO_2$, with only a small amount present from other aerosols. The average amount of $TiO_2$ present on the pad from the 50-liter chamber was 95.4% with a SD of 3 and a RSD of 3. The weights (mg) of $TiO_2$ were subsequently divided by the volume of air drawn through the pads (10 L total) to determine the individual aerosol concentrations ($mg/m^3$, column 7). For the dissemination of one grenade,

the mean concentration, SD and RSD for $TiO_2$ in the 500-L chamber were 1,808 mg/m$^3$, 149 and 8.2, respectively.

Percent recoveries and aerosol concentrations of $TiO_2$ were also calculated from collected samples following the dissemination of one grenade from the dissemination chamber (20,000-L chamber) and are shown in Table 9. The average amount of $TiO_2$ present on the pad was 94% with an sSD of 3 and RSD of 3. The mean concentration, SD and RSD for $TiO_2$ in the 20,000-L chamber were 4,230 mg/m$^3$, 160 and 3.8, respectively.

Table 8. $TiO_2$ Concentrations (mg/m$^3$) in 500 L Animal Exposure Chamber

| Date | Ti Pad Conc. (ppm) | Ti Pad wt (mg) | $TiO_2$ pad wt (mg) | Aerosol wt (mg) | $TiO_2$ on GFF (%) | $TiO_2$ conc (mg/m$^3$) |
|---|---|---|---|---|---|---|
| 7/10/2006 | 111.005 | 11.100 | 18.516 | 19.172 | 96.580 | 1851.625 |
| 7/10/2006 | 114.833 | 11.483 | 19.155 | 19.167 | 99.936 | 1915.474 |
| | | | | | | |
| 7/11/2006 | 102.392 | 10.239 | 17.080 | 18.577 | 91.940 | 1707.964 |
| 7/11/2006 | 105.263 | 10.526 | 17.559 | 18.384 | 95.510 | 1755.851 |
| | | | | | | |
| 7/12/2006 | 122.010 | 12.201 | 20.352 | 20.920 | 97.284 | 2035.191 |
| 7/12/2006 | 115.789 | 11.579 | 19.314 | 20.515 | 94.148 | 1931.436 |
| | | | | | | |
| 7/13/2006 | 98.565 | 9.856 | 16.441 | 16.920 | 97.170 | 1644.115 |
| 7/13/2006 | 97.129 | 9.713 | 16.202 | 17.838 | 90.827 | 1620.172 |

Table 9. $TiO_2$ Concentrations (mg/m$^3$) in 20,000-L Chamber

| Date | Ti Pad Conc. (ppm) | Ti Pad wt (mg) | $TiO_2$ pad wt (mg) | Aerosol Wt (mg) | $TiO_2$ on GFF (%) | $TiO_2$ (mg/m$^3$) |
|---|---|---|---|---|---|---|
| 7/10/2006 | 25.359 | 2.536 | 4.230 | 4.356 | 97.108 | 4230.005 |
| 7/11/2006 | 26.316 | 2.632 | 4.390 | 4.610 | 95.220 | 4389.628 |
| 7/12/2006 | 24.402 | 2.440 | 4.070 | 4.498 | 90.493 | 4070.382 |

The determination of $Al_2O_3$ and $SiO_2$ were not calculated directly through analysis of GFFs by AAS because the concentrations were below their respective detection limits of the instrument. The GFF pads themselves also had high levels of interferents making analysis difficult. Therefore, solid "fallout" material was collected from the 20,000-L chamber and analyzed via AAS to determine the percentage of $Al_2O_3$ and $SiO_2$ in the payload. The percentage of $Al_2O_3$ and $SiO_2$ found in the payload was determined to be 2.19 and 0.91, respectively. By using the percent composition of $Al_2O_3$ and $SiO_2$ found in the payload, the aerosol concentration for each oxide can be calculated. Table 10 gives the total aerosol weight collected on the pad and is provided in column 1. By multiplying the percent composition of each oxide with the total aerosol weight, the estimated weight of each oxide on the GFF pad can be calculated and are given in columns 3 and 4, respectively. Dividing the estimated weights of each oxide by the volume of air drawn, the individual aerosol concentrations for $Al_2O_3$ and $SiO_2$ are determined

and given in columns 5 and 6. The same calculation for the estimated aerosol calculations is performed on the GFF pad weights from the 20,000-L chamber for both oxides and given in Table 11.

For the disseminations of one grenade, the mean concentration, SD and RSD for $Al_2O_3$ were 41.4 mg/m$^3$, 2.9 and 6.9 in the 500-L chamber and 98.1 mg/m$^3$, 2.8 and 2.8 from the 20,000-L chamber. For the disseminations of one grenade, the mean concentration, SD and RSD for $SiO_2$ was 20.0 mg/m$^3$, 1.4 and 6.9 in the 500-L chamber and 47.4 mg/m$^3$, 1.3 and 2.8 from the 20,000-L chamber.

Table 10. $Al_2O_3$ Concentrations (mg/m$^3$) and $SiO_2$ Concentrations (mg/m$^3$) from 500-L Chamber

| Date | Aerosol Wt. (mg) | Wt of $Al_2O_3$ on Pad (mg) | Wt of SiO2 on Pad (mg) | $Al_2O_3$ conc (mg/m$^3$) | SiO2 conc (mg/m$^3$) |
|---|---|---|---|---|---|
| 7/10/2006 | 19.172 | 0.419 | 0.203 | 41.886 | 20.254 |
| | 19.167 | 0.419 | 0.202 | 41.875 | 20.249 |
| 7/11/2006 | 18.577 | 0.406 | 0.196 | 40.586 | 19.626 |
| | 18.384 | 0.402 | 0.194 | 40.164 | 19.422 |
| 7/12/2006 | 20.920 | 0.457 | 0.221 | 45.705 | 22.101 |
| | 20.515 | 0.448 | 0.217 | 44.820 | 21.673 |
| 7/13/2006 | 16.920 | 0.370 | 0.179 | 36.966 | 17.875 |
| | 17.838 | 0.390 | 0.188 | 38.971 | 18.845 |

Table 11. $Al_2O_3$ Concentrations (mg/m$^3$) and $SiO_2$ Concentrations (mg/m$^3$) from 20,000-L Chamber

| Date | Aerosol Wt. (mg) | Wt of $Al_2O_3$ on Pad (mg) | Wt of SiO2 on Pad (mg) | $Al_2O_3$ conc (mg/m$^3$) | SiO2 conc (mg/m$^3$) |
|---|---|---|---|---|---|
| 7/10/2006 | 4.356 | 0.095 | 0.0460 | 95.167 | 46.019 |
| 7/11/2006 | 4.610 | 0.101 | 0.0487 | 100.716 | 48.703 |
| 7/12/2006 | 4.498 | 0.098 | 0.0475 | 98.270 | 47.520 |

3.7 Observation of Toxic Signs.

Outward signs of respiratory distress during exposure were not observed during any of the $TiO_2$ exposures. Additionally, rats did not exhibit any signs of respiratory distress post-exposure and exhibited normal behavior.

3.8 BAL Evaluations.

For each test animal, the recovered lavage fluid was analyzed for total cell count, differential cell count and the presence of the pro-inflammatory cytokines, TNFα and IL1. Differential cell counts were divided into white blood cells (WBCs), pulmonary alveolar macrophages (PAMs), lymphocytes and polymorphonucleocytes (PMNs). Table 12 shows the 24-hr and 14-day post exposure data for the study groups. The final column shows a rating scale for cytoplasmic inclusion of the test material observed in the macrophages, where the grading used (0 – 4+) reflects the degree of cell particle burden.

The lung lavage analysis showed little evidence for an inflammatory response that would lead to toxicity. Total cell counts showed no significant pattern of change as compared to the control groups. Differential cell counts also showed non-specific increases in PMNs and PAMs, none of which could be correlated with an increase in exposure concentration or time post exposure. Group 12 did show a two fold increase in total cell count, which could provide evidence for a second influx of PAMS; however the increase was not significant as compared to the controls. At 24-hr post exposure, Group 11 had elevated PMN counts that were significantly different from Group 1, but at 14-day post exposure, the counts were reduced again. Both of these observations could suggest a mild pervasive inflammatory response associated with particle burden but the observations are inconsistent. Cytoplasmic inclusion of particulate $TiO_2$ was observed in all exposure groups. Lung lavage fluid analysis was unable to detect the presence of the pro-inflammatory cytokine TNFα or IL1 in the lavage fluid recovered from any control animal or any animal from any exposure concentration or time post exposure group.

3.9 Histopathological Evaluations.

The lung tissues were analyzed for histopathological changes. Representative lung tissue samples are depicted in Figures 11-14. Table 13 summarizes the sign and symptom categories along with the number of test animals that exhibited those signs.

Accumulation of small amounts of dark brown to black, and occasionally slightly refractile, pigment was observed within macrophages scattered throughout sections of lung examined from all $TiO_2$ exposed groups. A similar intrahistiocytic pigment accumulation was not observed in any of the control animals, therefore, the accumulation of pigment was considered to be a test article related alteration. Remarkable differences in the amount or character of pigment accumulation were not observed between exposure groups. While the pigment accumulation was test article related, there was no associated inflammation, necrosis, or other histological alterations in conjunction with the pigment, and therefore, the pigment was considered to be toxicologically insignificant. Non-specific mild subacute inflammation was observed in the control and exposure groups.

Table 12. 24-hr and 14-day Post Exposure BAL Results

| Exposure Group | ID # | Vol. Rec. | Recovery Percentage | Total Cells ($x10^4$) | WBC ($x10^3$) | Mac % | Lym % | PMN % | Cyto Incl.* |
|---|---|---|---|---|---|---|---|---|---|
| Air Control (Group 1) 24 Hr PE | 11 | 14.5 | 80.6 | 1.8 | 1.3 | 96 | 4 | 0 | 0 |
| | 13 | 6.5 | 36.1 | 6.9 | 1.8 | 97 | 3 | 0 | 0 |
| | 14 | 15.0 | 83.3 | 3.0 | 3.4 | 95 | 5 | 0 | 0 |
| | 15 | 13.5 | 75.0 | 1.5 | 0.3 | 77 | 21 | 0 | 0 |
| | 18 | 15.0 | 83.3 | 1.6 | 1.0 | 96 | 4 | 0 | 0 |
| | Mean | 12.9 | 71.7 | 2.6 | 2.6 | 92 | 7 | 0 | |
| | SD | 3.6 | 20.2 | 1.8 | 1.3 | 9 | 8 | 0 | |
| Air Control (Group 2) 14 Days PE | 32 | 15.0 | 83.3 | 3.9 | 9.3 | 97 | 3 | 0 | 0 |
| | 33 | 13.0 | 72.2 | 3.6 | 8.5 | 98 | 1 | 1 | 0 |
| | 38 | 14.0 | 77.8 | 3.2 | 3.2 | 96 | 4 | 0 | 0 |
| | 39 | 13.0 | 72.2 | 2.2 | 4.5 | 97 | 3 | 0 | 0 |
| | 40 | 16.0 | 88.9 | 1.3 | 3.8 | 96 | 3 | 1 | 0 |
| | Mean | 14.2 | 78.9 | 2.8 | 5.9 | 96.8 | 2.8 | 0.4 | |
| | SD | 1.3 | 7.2 | 1.0 | 2.8 | 0.8 | 1.1 | 0.5 | |
| Acute 1 grenade (Group 3) 24 Hr PE | 26 | 13.0 | 72.2 | 2.3 | 2.7 | 96 | 4 | 0 | 2+ |
| | 27 | 13.0 | 72.2 | 5.6 | 0.6 | 99 | 1 | 0 | 1+ |
| | 28 | 14.0 | 77.8 | 2.7 | 2.8 | 98 | 2 | 0 | 2+ |
| | 29 | 15.0 | 83.3 | 1.1 | 4 | 99 | 1 | 0 | 2+ |
| | 30 | 14.0 | 77.8 | 1.3 | 3.4 | 95 | 5 | 0 | 2+ |
| | Mean | 13.8 | 76.7 | 2.6 | 2.7 | 97 | 3 | 0 | |
| | SD | 0.8 | 4.6 | 1.8 | 1.3. | 2 | 2 | 0 | |
| Acute 1 grenade (Group 4) 14 Days PE | 9 | 15.0 | 83.3 | 8.0 | 5.4 | 100 | 0 | 0 | 2+ |
| | 31 | 17.5 | 72.9 | 2.3 | 6.5 | 98 | 1 | 1 | 3+ |
| | 34 | 16.0 | 88.9 | 2.0 | 3.2 | 98 | 2 | 0 | 1+ |
| | 36 | 15.0 | 83.3 | 1.3 | 3.3 | 97 | 3 | 0 | 3+ |
| | 42 | 14.0 | 77.8 | 2.5 | 9.8 | 97 | 3 | 0 | 0 |
| | Mean | 15.5 | 81.3 | 3.2 | 5.6 | 98.0 | 1.8 | 0.2 | |
| | SD | 1.3 | 6.1 | 2.7 | 2.7 | 1.2 | 1.3 | 0.4 | |
| Repeat 1 Grenade 2 Days (Group 5) 24 Hr PE | 47 | 15.0 | 83.3 | 1.2 | 1.8 | 94 | 5 | 1 | 1+ |
| | 51 | 14.0 | 77.8 | 1.4 | 2 | 96 | 3 | 1 | 3+ |
| | 54 | 14.0 | 77.8 | 3.2 | 1.7 | 98 | 2 | 0 | 1+ |
| | 55 | 16.0 | 88.9 | 3.7 | 5.1 | 98 | 2 | 0 | 1+ |
| | 56 | 14.0 | 77.8 | 1.3 | 3.3 | 95 | 5 | 0 | 1+ |
| | Mean | 14.6 | 81.1 | 2.2 | 2.8 | 96 | 3 | 0 | |
| | SD | 0.9 | 5.0 | 1.2 | 1.4 | 2 | 2 | 1 | |
| Repeat 1 Grenade 2 Days (Group 6) 14 Days PE | 58 | 14.0 | 77.8 | 2.2 | 5.5 | 98 | 2 | 0 | 4+ |
| | 60 | 14.0 | 77.8 | 1.1 | 1.7 | 92 | 6 | 2 | 4+ |
| | 61 | 14.0 | 77.8 | 1.3 | 2.4 | 96 | 4 | 0 | 4+ |
| | 63 | 12.5 | 69.4 | 2.3 | 3 | 96 | 4 | 0 | 4+ |
| | 67 | 14.0 | 77.8 | 1.9 | 1.6 | 93 | 4 | 3 | 4+ |
| | Mean | 13.7 | 76.1 | 1.8 | 2.8 | 95 | 4 | 1 | |
| | SD | 0.7 | 3.7 | 0.5 | 1.6 | 2 | 1 | 1 | |

Table 12. 24-hr and 14-day Post Exposure BAL Results (continued)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Repeat 1 Grenade 4 Days (Group 7) 24 Hr PE | 68 | 15.0 | 83.3 | 2.0 | 2.7 | 94 | 5 | 1 | 4+ |
| | 69 | 15.0 | 83.3 | 3.2 | 6.3 | 96 | 3 | 1 | 4+ |
| | 70 | 16.0 | 88.9 | 1.3 | 2.2 | 95 | 3 | 2 | 4+ |
| | 71 | 16.0 | 88.9 | 2.8 | 4.2 | 99 | 1 | 0 | 4+ |
| | 72 | 17.0 | 94.4 | 1.8 | 2.3 | 99 | 0 | 1 | 4+ |
| | Mean | 15.8 | 87.8 | 2.2 | 3.5 | 97 | 2 | 1 | |
| | SD | 0.8 | 4.6 | 0.8 | 1.7 | 2 | 2 | 1 | |
| Repeat 1 Grenade 4 Days (Group 8) 14 Days PE | 79 | 12.0 | 66.7 | 0.9 | 1.6 | 95 | 5 | 0 | 4+ |
| | 80 | 14.0 | 77.8 | 1.0 | 2.5 | 100 | 0 | 0 | 4+ |
| | 81 | 15.0 | 83.3 | 1.2 | 2.7 | 98 | 2 | 0 | 3+ |
| | 83 | 15.0 | 83.3 | 0.8 | 0.9 | 97 | 2 | 1 | 4+ |
| | 84 | 15.0 | 83.3 | 1.0 | 1.0 | 97 | 3 | 0 | 4+ |
| | Mean | 14.2 | 78.9 | 1.0 | 1.7 | 97 | 2 | 0 | |
| | SD | 1.3 | 7.2 | 0.1 | 0.8 | 2 | 2 | 0 | |
| Acute 2 Grenades (Group 9) 24 Hr PE | 92 | 17.5 | 97.2 | 1.9 | 1.3 | 100 | 0 | 0 | 4+ |
| | 100 | 17.0 | 94.4 | 3.6 | 3.0 | 96 | 4 | 0 | 4+ |
| | 101 | 17.5 | 97.2 | 0.8 | 1.1 | 97 | 2 | 1 | 4+ |
| | 103 | 17.5 | 97.2 | 1.4 | 0.9 | 97 | 3 | 0 | 4+ |
| | 107 | 18.0 | 100.0 | 3.8 | 5.0 | 95 | 5 | 0 | 4+ |
| | Mean | 17.5 | 97.2 | 2.3 | 2.3 | 97 | 3 | 0 | |
| | SD | 0.4 | 2.0 | 1.3 | 1.7 | 2 | 2 | 0 | |
| Acute 2 Grenades (Group 10) 14 Days PE | 108 | 14.0 | 77.8 | 1.4 | 9.2 | 97 | 2 | 1 | 4+ |
| | 110 | 14.0 | 77.8 | 2.5 | 12.3 | 96 | 3 | 1 | 4+ |
| | 115 | 14.5 | 80.6 | 2.5 | 10.0 | 96 | 4 | 0 | 4+ |
| | 118 | 14.5 | 80.6 | 3.3 | 14.7 | 97 | 3 | 0 | 4+ |
| | 120 | 15.0 | 83.3 | 3.2 | 14.8 | 100 | 0 | 0 | 4+ |
| | Mean | 14.4 | 80.0 | 2.6 | 12.2 | 97 | 2 | 0 | |
| | SD | 0.4 | 2.3 | 0.8 | 2.6 | 2 | 2 | 1 | |
| Repeat 2 Grenades 4 Days (Group 11) 24 Hr PE | 112 | 17.0 | 94.4 | 2.5 | 3.9 | 90 | 6 | 4 | 4+ |
| | 114 | 17.5 | 97.2 | 1.7 | 3.4 | 93 | 5 | 2 | 4+ |
| | 116 | 17.0 | 94.4 | 3.5 | 4.1 | 90 | 7 | 3 | 4+ |
| | 117 | 14.5 | 80.6 | 2.6 | 6.8 | 97 | 2 | 1 | 4+ |
| | 119 | 17.5 | 97.2 | 2.3 | 6.0 | 96 | 2 | 2 | 4+ |
| | Mean | 16.7 | 92.8 | 2.5 | 4.8 | 93 | 4 | 2 | |
| | SD | 1.3 | 7.0 | 0.6 | 1.5 | 3 | 2 | 1 | |
| Repeat 2 Grenades 4 Days (Group 12) 14 Days PE | 109 | 15.5 | 86.1 | 5.6 | 3.4 | 98 | 2 | 0 | 4+ |
| | 127 | 15.0 | 83.3 | 7.1 | 33.0 | 99 | 1 | 0 | 4+ |
| | 131 | 16.0 | 88.9 | 6.1 | 37.6 | 97 | 3 | 0 | 4+ |
| | 132 | 16.5 | 91.7 | 1.6 | 2.5 | 100 | 0 | 0 | 4+ |
| | 134 | 15.0 | 83.3 | 4.4 | 11.9 | 99 | 1 | 0 | 4+ |
| | Mean | 15.6 | 86.7 | 5.0 | 17.7 | 99 | 1 | 0 | |
| | SD | 0.7 | 3.6 | 2.1 | 16.6 | 1 | 1 | 0 | |

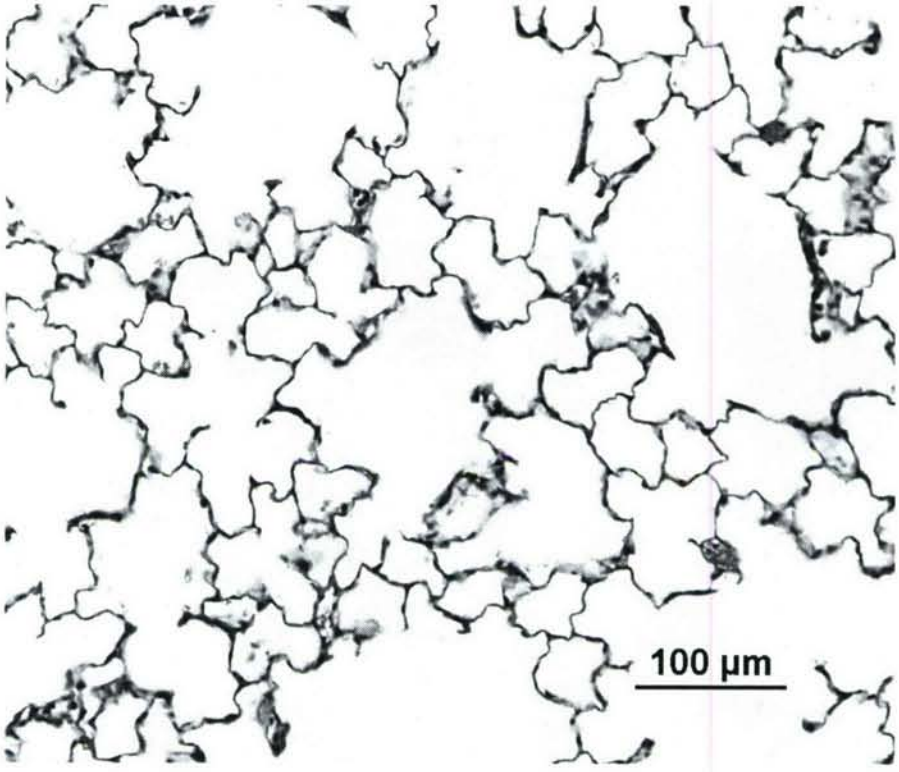


Figure 11. Normal Lung Tissue HE 20X

Figure 12. PAMs with $TiO_2$ HE 40X. Group 6 (2-Day Repeat, 1 Grenade, 14-Day Post Exposure)

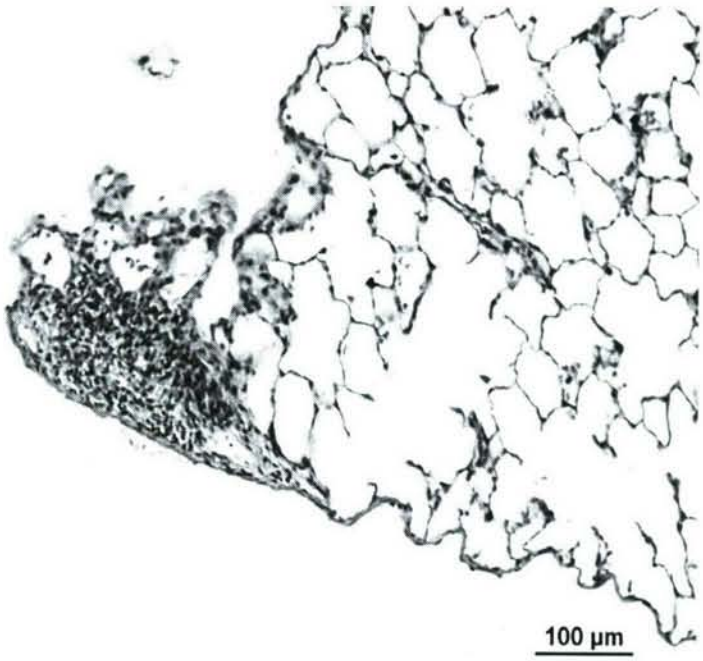


Figure 13. PAM Infiltration HE 20X. Group 11 (4 –Day Repeat, 2 Grenades, 24 hr Post Exposure)

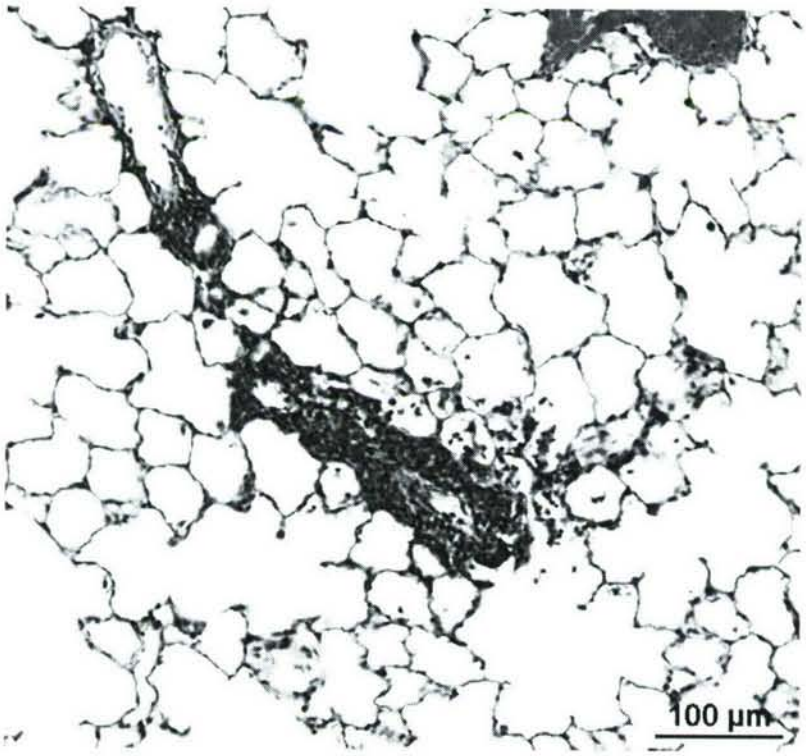


Figure 14. Peribronchiolar Inflammation in Lung Tissue HE 20X. Group 5 (2-Day Repeat, 1 Grenade, 24-hr Post Exposure)

Table 13. 24-hr and 14-day Post Exposure Histopathological Results

| Signs and Symptoms | Grp 1 | Grp 2 | Grp 3 | Grp 4 | Grp 5 | Grp 6 | Grp 7 | Grp 8 | Grp 9 | Grp 10 | Grp 11 | Grp 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alveolar Macrophages | 0/5 | 1/5 | 1/5 | 1/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| Infiltration of PMN | 4/5 | 4/5 | 4/5 | 4/5 | 3/5 | 4/5 | 0/5 | 5/5 | 4/5 | 3/5 | 5/5 | 3/5 |
| Fibrosis minimal | 0/5 | 0/5 | 0/5 | 1/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| Hemorrhage, minimal | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| Hyperplasia, bronch. mild | 2/5 | 0/5 | 2/5 | 2/5 | 1/5 | 0/5 | 0/5 | 2/5 | 1/5 | 2/5 | 1/5 | 0/5 |
| Hyperplasia, TypeII mild | 1/5 | 1/5 | 1/5 | 5/5 | 2/5 | 3/5 | 2/5 | 1/5 | 2/5 | 2/5 | 3/5 | 0/5 |
| $TiO_2$ Laden Macrophages | 0/5 | 0/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| Inflammation, mild | 1/5 | 1/5 | 1/5 | 2/5 | 1/5 | 2/5 | 0/5 | 1/5 | 2/5 | 2/5 | 2/5 | 0/5 |

Grp 1 = Air Control (24 hrs. PE), Grp 2 = Air Control (14 days PE)
Grp 3 = Acute 1 grenade (24 hr PE),Grp 4 = Acute 1 grenade (14 days PE)
Grp 5 = 2-day repeat 1 grenade (24 hr PE), Grp 6 = 2-day repeat 1 grenade (14 days PE)
Grp 7 = 4-day repeat 1 grenade (24 hr PE), Grp 8 = 4-day repeat 1 grenade (14 days PE)
Grp 9 = Acute 2-grenades (24 hr PE), Grp 10 = Acute 2-grenades (14 days PE)
Grp 11 = 4-day repeat 2 grenades (24 hr PE),Grp 12 = 4-day repeat 2 grenades (14 days PE)

## 4. DISCUSSION

### 4.1 Exposure Concentrations.

Although, stricter controls are able to be exerted than for field dissemination experiments, variance is still introduced by the amount of total particulate material produced from the FOG(s) in the 20,000-L chamber. Within the same lot number, differences still occur in the yields produced. Initially, exposure concentrations were chosen to simulate the concentration that would be achieved if one FOG device were disseminated in a 12' x 12' x 12' room (1728 $ft^3$ ≅ 50,000 L) or similar confined location. For one FOG device, the mean total aerosol concentration in the 20,000-L chamber was 4,211 $mg/m^3$. At the time of sampling, this equates to 33% of the initial payload becoming airborne. Since our chamber is approximately 40% of the volume in comparison to the air volume that would be contained within a 12' x 12' x 12', the target concentration for the initial animal exposures should be 2 ½ times less than 4,211 $mg/m^3$ or approximately 1,684 $mg/m^3$. In the current study, when the smoke was diverted from the 20,000-L chamber to the 500-L chamber, the actual exposure concentrations were 1,700 $mg/m^3$, 1774 $mg/m^3$ and 1894 $mg/m^3$ for the acute, 2-day repeat and 4-day repeat exposures. Therefore,

the experimentally determined concentrations were in excellent accordance and slightly higher than the desired target value of 1,684 mg/m$^3$.

Additional exposures were conducted where two FOGS were disseminated simultaneously in the 20,000-L chamber. Scenarios might occur where more than one FOG device is used in a small area. Based on the previous concentrations, if one FOG device should theoretically produce 1,684 mg/m$^3$ within a 12 ' x 12' x 12' room, then two devices disseminated simultaneously should produce approximately 3,360 mg/m$^3$. Experimentally, the acute concentration for animal exposures in our 500-L chamber was 3,697 mg/m$^3$ and the 4-day repeat concentration was 3,638 mg/m$^3$. Both are within 10% of the desired target concentration.

## 4.2 Particle Size

Small particle sizes are not uncommon for disseminated materials that are pyrotechnically generated. Some items that have been previously tested are accommodated with elevated temperatures, thereby producing extremely small particles (i.e., MMAD 's < 1.0μm). This has been observed previously during chemical characterization tests performed on red phosphorous items, where flaming occurs during the dissemination.[15,16] FOG devices are intended to be used in smaller, confined environments where flaming would be unfavorable and unsafe. In the current configuration, the FOGs are not accompanied with the extreme temperatures that are observed for red phosphorous smokes; therefore, the FOGs do not flame during dissemination.

The mean MMAD for one FOG dissemination was 2.24 μm. This implies that the particles will likely undergo impaction in the alveolar portion of the lower respiratory system.[17] With this particle size, it is likely that diffusion mechanisms at the blood barrier will not occur but deep deposition into the respiratory system is still probable. Further evidence is provided by the high mean percentage (70%) of particles that were found to be respirable (< 3 μm). Respirable is defined by the ACGIH as particles that are deposited in the gas-exchange alveolar region of the lung.

The MMAD for two FOG disseminations was 3.17μm. With the dissemination of two items simultaneously, the possibility increases for the formation of aggregates. There is some preliminary evidence to support this with the current study. With larger particles forming, the percentage that are respirable should also decrease. Only a small decrease was observed in the current study as the respirable percentage fell slightly from 70 to 63%. Some deposition though might begin to occur at locations further up the respiratory system, away from the alveolar portions of the lungs. Bimodal distribution was not observed in the current study.

## 4.3 VOCs.

Because the original $TiO_2$ smoke payload did not contain substantial portions of it's fill from organic materials, it was not expected to produce VOC concentrations of toxicological significance. This is in contrast to the M8 smoke pot and M83 grenade, which contain terephthalic acid as their principal component. Chemical characterization studies have shown benzene and formaldehyde production during the dissemination of these items that were

above their respective TLV-TWA's.[18,19,20,21] The FOG device is a burster grenade where formation of VOCs would only occur at the time of dissemination, but then quickly dissipate. This is in contrast to burning grenades, where the production of smoke occurs over a longer period of time. Generation of VOCs could be produced during the entire dissemination process and could possibly be more likely to be detected.

Long chained aliphatic alkanes (>20 carbons) are compounds traditionally seen from the dissemination and combustion of smoke materials. To separate these compounds by GC/MS, elevated temperatures were necessary for the movement of these compounds through the analytical column and their subsequent identification with mass selective detection. Identification of the individual aliphatic compounds was confirmed by the distinctive fragmentation patterns observed. Typically, straight chain alkanes are rather simple to elucidate from the consistent loss of 14 atomic mass units (amu's). This weight loss corresponds to the loss of subsequent methylene (-CH2) groups in the compounds. Quantitation of these hydrocarbon compounds along with other smaller hydrocarbons was unnecessary because their concentrations were not of toxicological significance.

If the same VOCs were produced during the disseminations, it would be expected to detect the compounds during all of the analyses. Samples were taken during the duration of the 10-min exposures for 1 lpm. With the fast flow sorbent tubes that were used, this flow rate represented the highest possible flow that could be used without risking breakthrough of any materials. If compounds would have been present that would have been considered toxicologically significant, they would have consistently appeared. No polyaromatic hydrocarbons were detected in the disseminated smoke.

4.4 Inorganic Gases.

Following disseminations, air samples were analyzed for inorganic gases that are considered to be of toxicological concern. Table 14 lists the Threshold Limit Values-Time Weighted Averages (TLV-TWA'S) and the toxicological effect for the inorganic gases found. The values are as listed by the ACGIH.[22] CO was observed during the disseminations but did not exceed its established TLV. The $NO_x$ tubes are an EPA standard that simultaneously measures for nitric oxide (NO) and nitrogen dioxide ($NO_2$). During combustion processes, the primary pollutant is the free radical form of NO. Usually, conversion occurs in tens of minutes to $NO_2$. By the way in which the experiment was designed, most of the $NO_x$ was probably in the form of $NO_2$ by the time the air was analytically sampled. The highest concentrations of $NO_x$ and $CO_2$ were 0.25 ppm and 350 ppm, respectively. Both of these values are much lower than the established ACGIH regulatory limits.

Table 14. TLV-TWA and Toxicological Effects for Inorganic Gases

| Anion/Gas | TLV-TWA (ppm) | Effect |
|---|---|---|
| NO | 25 | irritation |
| $NO_2$ | 3 | irritation |
| CO | 25 | Anoxia, *CVS,**CNS |
| $CO_2$ | 5000 | Asphyxiation |

*CVS – CardioVascular System
**CNS – Central Nervous System

4.5 Metal Analysis.

For the dissemination of one or two FOGs, all of the metal oxides observed during the study greatly exceeded the threshold values that are currently established by the ACGIH (Table 15). All of the metal oxides observed were expected as they were all contained in the original smoke fill. Most of the problems inherently seen with the inhalation of metallic dusts appear from the continual, chronic inhalation of these materials. Generally, the smaller a particle's aerodynamic diameter, the greater the probability it will penetrate and deposit in the distal (lower) portions of the respiratory tract. Although the presence of metal oxides was detected among the particles, the histopathological and BAL evaluations did not present evidence that a toxicological response was present (Section 4.6).

Table 15. TLV-TWA and Toxicological Effects for Metal Oxides

| Metal Oxide | TLV-TWA ($mg/m^3$) | Effect |
|---|---|---|
| Titanium Dioxide | 10 | Lower respiratory irritant |
| Aluminum Oxide | 10 | Lower respiratory irritant, |
| Silica, Crystalline | 0.05-1 | Lung fibrosis, silicosis |

4.6 BAL and Histopathological Evaluations.

The $TiO_2$ is a relatively non-toxic ceramic dust material that is commonly used as a negative control in inhalation toxicity studies.[23] Exposure to high concentrations of $TiO_2$, 6 hr exposures, 5 days a week for four consecutive weeks to 250 $mg/m^3$, have been linked with persistent inflammatory responses and dust overload phenomena.[23,24] The exposure concentrations generated in this study through pyrotechnic generation of a $TiO_2$ atmosphere were extremely high, as much as 4,211 $mg/m^3$, but the exposure duration was short (10-min exposures repeated once a day for up to 4 days). Efforts were therefore made to quantify the toxicological response to these exposure conditions.

The BAL and histopathological evaluations conducted in this study (Tables 12 and 13), indicate that some data suggests an inflammatory response, but the results are inconclusive. It can be expected that inhalation of this environment for significantly longer durations or more repeat exposures could eventually lead to lung burdens great enough to overload normal clearance mechanisms and build up a toxicological response; however, the transient nature of the pyrotechnically generated cloud during the current study led to short exposure durations and therefore limited any potential toxicological response.[24] The histopathological and BAL evaluations indicated that a normal macrophage mediated dust clearance phenomena was incomplete at two weeks post exposure. Previously a clearance half-life of 53 days after a single 7-hr exposure to 19 $mg/m^3$ has been reported, so it is not unexpected that clearance was incomplete at 14 days post exposure.[25]

## 5. CONCLUSIONS

FOG burster grenades were pyrotechnically disseminated to determine the inhalation toxicology effects of $TiO_2$ smoke. Sprague-Dawley rats were exposed to high concentrations (up to 4000 $mg/m^3$) for short periods of time (10 min) either acutely or repeatedly for up to four consecutive days. Particle size analysis revealed that over 60% of the pyrotechnically disseminated material was respirable and would therefore be deposited deep within the respiratory system. Aerosol characterization of the metals also confirmed that the predominant portion of the aerosol (>95%) was comprised of $TiO_2$ with smaller percentages of $Al_2O_3$ and $SiO_2$. All three of these oxides significantly exceeded their respective regulatory limits as published by the ACGIH. No inorganic gas or VOC concentrations were observed of toxicological significance.

Several observations from histopathological examination of lung tissues suggest a mild inflammatory response to inhalation of test material. Focal infiltration of inflammatory cells was observed in some tissue sections to include both peripheral accumulation of cells, which is likely the result of collection and accumulation of particle laden PAMs in the lymphatic drainage; and evidence of peribronchiolar accumulation of PAMs accompanied by separation and sloughing of bronchiolar epithelial tissue. However, most observations of accumulation of PAMs and other inflammatory cells were not accompanied by evidence of tissue injury or effacement of alveolar septa. Evidence that cell injury had occurred is suggested by the observation of Type II cell hyperplasia indicating a subsequent replacement of damaged type I pneumocytes as part of the normal injury repair process. Elevation of PAMs generally was not reported following the histopathological examination; yet elevation of PMNs was reported for nearly all of the exposure groups. An influx of PMNs into the lumen usually occurs in conjunction with or following the recruitment and infiltration of PAMs, which are a source of chemotractant stimulating PMN recruitment. Particle and or pigment laden PAMs were observed in the tissue sections. The histopathological results are equivocal in that many of the observations indicative of an inflammatory response and subsequent repair also were present in control animals at nearly the same or even higher incidence rate. Likewise some of the histopathological observations may be subject to further interpretation. For example examination of the type and relative number of free cells in the lung lumen is difficult to achieve from histopathological preparations, particularly when methods involving intralumenal fixation

of the tissues under pressure are used. Clustering and aggregation of PAMs and free cells in the lumen and alveolar acini could be an artifact of tissue fixation process. In addition, cells not adhered to lung tissue are likely to be lost during preparation for sectioning making it difficult to observe sufficient numbers of cells to perform accurate differential analysis, unless the recruitment and influx of inflammatory cells is extraordinarily heavy, for which there is no evidence in the present study.

A more direct and definitive assay of lung free cell population and type is obtained from analysis of the BAL. BAL analysis did not show an exposure related increase in PAMs except in the repeated exposure regimen, which resulted in the highest collective particle exposure. In this group, the total cell count BAL at 14 days post exposure was nearly twice that for controls. A similar elevation in total cell count was not observed 24 hr post exposure for this exposure level. Either recruitment of PAMs was not complete this soon after exposure or there was a second influx of PAMs, as has been shown to occur for clearing debris resulting from an initial recruitment of PAMs in response to particle deposition. A slight elevation of PMNs was observed in the highest total exposure concentration group 24 hr following the last exposure; however, at 14 days, the proportion of PMNs in the total cell population had returned to normal, indicating that the initial inflammatory response sufficient to elicit an influx of PMNs was not persistent. Other indicators of inflammation in the BAL did not suggest a substantial inflammatory response. Neither of the proinflammatory cytokines TNF$\alpha$ nor IL-1 was elevated in the BAL. Particle burdens in PAMs harvested from BAL were proportional to the exposure level and particle laden PAMs could be seen 24 hr and 14 days post exposure – but with no apparent difference in either number of laden PAMs or the magnitude of particle burden within individual PAMs as a function of post exposure time. This observation is consistent with normal PAM mediated particle clearance from the alveolar acini, which may take several months to complete.

When analyzed collectively, histopathological examination of lung tissues and analysis of the BAL indicate a test material induced inflammatory response that is focal, of inconsequential severity, and is most likely proportional to the total particle burden delivered by the exposures. However, there are no indications that the observed inflammatory response even at the heaviest of particle burdens is sufficiently severe or sustained to lead to toxicological responses characterized by abnormal restructuring of lung tissue or respiratory dysfunction.

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